FBS05- Acid Phosphatase Presumptive Chemical Test for the Presence of Seminal Fluid

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1. Scope

1.1. This procedure is used to determine the possible presence of seminal fluid on evidentiary material.

2. Background

2.1. Qualitative acid phosphatase (AP) testing is used as a screening test for seminal fluid. α–Naphthyl phosphate is acted upon by the enzyme AP to produce α–naphthol, which then combines with diazo blue B dye to form a violet colored complex. In the absence of AP, α–naphthyl phosphate is not able to combine with the diazo blue B compound and the reaction will remain colorless.

2.2. AP originates in the prostate gland. Although present in other body fluids, it occurs in seminal fluid at concentrations 20 to 400 times higher than that of other body fluids. Since AP is not exclusive to human seminal fluid, it can only be used as a presumptive test for the presence of seminal fluid.

3. Safety

3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures (SOPs).

3.2. Read Safety Data Sheets (SDSs) to determine the safety hazards for chemicals and reagents used in the SOPs.

4. Materials Required

4.1. Acid Phosphatase Reagent (FBR22)

4.2. Positive Control-Semen (FBR03)

4.3. Autoclaved deionized water (diH_2O)

4.4. Alternate Light Source (ALS) (Rofin Forensic Polilight PL400, Polilight-Flare Plus, or Polilight-Flare Plus 2) - Optional

5. Standards and Controls

5.1. The Positive and Negative Controls are tested prior to daily use. Record the results in the applicable Sample Tracking and Control Solutions (STACS) documentation.

5.2. A known seminal fluid dilution strip, containing a 1:16 seminal fluid dilution and a 1:32 seminal fluid dilution, is tested as a Positive Control (FBR03). Apply the AP working solution directly to the seminal fluid dilution strip. This control should develop a violet/purple color upon addition of the AP working solution in the following time frames:

Positive Control Sample	Results
Seminal fluid - 1:16 dilution	Rapid violet/purple color (~0 to 10 seconds)
Seminal fluid – 1:32 dilution	Slow violet/purple color (~20 to 30 seconds)

5.3. An unstained area on the known seminal fluid dilution strip is tested as a Negative Control or Blank. This control should not exhibit a violet/purple color within 60 seconds upon addition of AP working solution.

6. Procedures

6.1. Stain areas may be located by a visual examination and/or with the aid of an Alternate Light Source (ALS).

6.2. Stain areas may be sampled using various methods:

6.2.1. Swab stain area with moistened swab (diH_2O)

6.2.2. Small cutting placed on filter paper or other appropriate clean surface

6.2.3. Press a damp filter paper (diH_2O) on the stain area (overlay) and maintain good contact for at least 3 seconds.

6.2.3.1. Remove the filter paper from the stain area

6.3. Add one drop of the AP working solution to the cutting, swab or filter paper. For large filter paper overlays, the reagent may be sprayed onto the filter paper or multiple drops may be added.

6.4. Using a timer, observe for 1 minute. A rapid color change to purple within 30 seconds is a presumptive positive result for the presence of seminal fluid. A color change from 31-59 seconds is an inconclusive result indicating the possible presence of seminal fluid. No color change at or after 60 seconds is a presumptive negative result for the presence of seminal fluid. Record the results in the applicable STACS documentation.

7. Sampling

7.1. Not applicable

8. Calculations

8.1. Not applicable

9. Uncertainty of Measurement

9.1. Not applicable

10. Limitations

10.1. The AP test is a presumptive test for the presence of seminal fluid. In order to confirm the presence of semen/seminal fluid, a microscopic examination for the presence of spermatozoa (FBS07) and/or a p30 test (FBS06) must be performed with a positive result.

10.2. A color change must be observed within 30 seconds to be considered a positive test result. An unlimited detection time could lead to a false positive reaction.

10.3. This test is dependent upon the amount of AP present in the sample. An inconclusive or negative presumptive test for the presence of AP does not necessarily mean that the stain tested does not contain seminal fluid. It is possible to have seminal fluid present in such dilute amounts in a stain that a positive reaction is not obtained. Additionally, AP is known to breakdown in the presence of heat and moisture or due to age of the stain, therefore there could

be little or no enzyme activity for these types of seminal fluid stains. Alternatively, a positive presumptive test for AP does not confirm the presence of seminal fluid.

10.4. Insufficient sample quality and/or quantity could limit the development of a positive reaction.

11. Documentation

11.1. Applicable STACS documentation

11.2. FBU Report of Examination

12. References

12.1. Acid Phosphatase Reagent (FBR22)

12.2. Positive Control – Seminal fluid (FBR03)

12.3. Use of Alternate Light Source to Aid in Stain Identification (FBS04)

12.4. P30 Antigen Test for the Presence of Seminal fluid (FBS06)

12.5. Microscopic Examination for the Presence of Spermatozoa by Christmas Tree Stain (FBS07)

12.6. Forensic Biology Unit Quality Assurance Manual